Trigeminal and cervical sensitization during the four phases of the migraine cycle in patients with episodic migraine

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ABSTRACT

Objective

Assessing mechanical pain thresholds from trigeminal, cervical, and distal pain-free areas during the 4 phases of a migraine cycle in episodic migraine patients (EM).

Methods

This multicenter, cross-sectional, observational study conducted in Parma and Genova's Headache Center assessed quantitative sensory tests during the 4 migraine phases in EM patients compared to controls. Temporal summation of pain (TSP), static pressure pain threshold (sPPT)), and mechanical pinprick pain threshold (MPT) were assessed from the trigeminal area, sPPT and dynamic PPT (dPPT) from the cervical area, sPPT and MPT over the hand, and sPPT from the tibialis anterior.

Results

A total of 135 patients and 46 controls were included. TSP was facilitated in ictal EM (EM versus controls: mean (SD) 2.7(2.0) versus 1.4(1.8); p=0.004); temporal sPPT and MPT were reduced in interictal (sPPT: 198.5(79.3) kPa; p=0.021; MPT: 12.6(15.7) g; p=0.001), preictal (sPPT: 200.6(71.6) kPa; p=0.033; MPT: 10.7(12.4) g; p<0.001), ictal (sPPT: 171.4(95.9) kPa; p<0.001; MPT: 7.3(12.0) g; p<0.001), and postictal EM (sPPT: 182.2(76.3) kPa; p=0.006; MPT: 10.1(14.9) g; p=0.001), compared to controls (sPPT: 238.3(73.8) kPa; MPT: 21.9(17.3) g). Cervical sPPTs and dPPT were reduced in interictal (sPPT upper cervical spine: 420.5(176.7) kPa; p=0.031; sPPT lower cervical spine: 458.6(207.3) kPa; p=0.002; dPPT: 4826.5(2698.0) g; p<0.001), preictal (sPPT upper cervical spine: 389.3(133.4) kPa; p=0.006; sPPT lower cervical spine: 450.8(174.3) kPa; p=0.005; dPPT: 4184.2(2628.3) g; p<0.001), ictal (sPPT upper cervical spine: 379.9(205.6) kPa p=0.003; sPPT lower cervical spine: 436.3(271.1) kPa; p=0.001; dPPT: 3838.3(2638.7) g; p<0.001), and postictal EM (sPPT upper cervical spine: 385.5(131.6) kPa; p=0.020; sPPT lower cervical spine: 413.0(150.3) kPa; p=0.002; dPPT: 4679.6(2894.9) g; p=0.001), compared to controls (sPPT upper cervical spine: 494.9(171.5) kPa; sPPT lower cervical spine: 586.9(210.8) kPa; dPPT: 7693.9

(2896.8) g). Preictal EM had reduced hand sPPT and MPT (sPPT: 248.8 (96.6) kPa versus 319.8(112.3) kPa; p=0.006; MPT: 23.6(12.2) g versus 32.5(14.4) g; p=0.035), while EM in the other phases showed reduction in hand MPT (interictal: 22.3(15.6) g versus 32.5(14.4) g; p=0.002; ictal: 22.4(17.0) g versus 32.5(14.4) g; p=0.004; postictal: 24.2(18.8) g versus 32.5(14.4) g; p=0.003) without significant reduction in hand sPPT. No difference in sPPT over the tibialis anterior was found. Hand MPT was negatively correlated with longer disease duration (r=-0.25; p=0.011) and hand sPPT was negatively correlated with higher drug usage (r=-0.31; p=0.002). TSP during the ictal phase was positively correlated with the physical (r=0.38; p=0.040) and emotional headache-related disability (r=0.53; p=0.003).

Conclusion

In all phases of the migraine cycle, EM patients show signs of sensitization in the trigeminocervical area, with patients with the most prominent sensitization in the ictal phase. Signs of widespread sensitization were consistent in preictal EM patients and in the subgroups of EM patients with the longest disease duration and more usage of symptomatic drugs.

INTRODUCTION

Migraine is a complex brain disorder characterized by cyclic changes in the excitability of cortical, subcortical, and brainstem areas[1,2]. Quantitative sensory testing (QST) can be applied in clinical and research settings[3], and reduced pain thresholds in the trigeminal, cervical, and distal pain-free areas have been used as proxies to assess sensitization in patients with migraine[4–6]. QST studies applied in episodic migraines (EM) provide evidence of cyclic changes in the pain thresholds in trigeminal, cervical, and pain-free areas[7–11]. Reduction in pain thresholds begins in the preictal phase, reaches its peak in the ictal phase, and lasts after resolution[7–11]. It is still uncertain if signs of trigeminal, cervical, and widespread sensitizations are also present in the interictal phase of the migraine cycle[12]. Most EM patient QST studies performed in the interictal phase do not control the proximity to the next headache attack[13], so data across studies are difficult to compare[12,14]. Data controlling

for the distance from previous and follow headache attacks suggested are no differences in electrical or thermal pain thresholds assessed from the trigeminal, cervical, and pain-free areas between EM patients in the interictal phase and healthy control[7,15–22]. On the other hand, mechanical pain threshold assessments in trigeminal and pain-free areas have shown both reduced trigeminal threshold in interictal EM compared to healthy controls[23], no difference in thresholds from the trigeminal and a distal pain-free area[19,22], or increased trigeminal threshold[24]. More data are needed to understand if sensitizations in trigeminal, cervical, and distal pain-free areas are general features in all phases of the migraine cycle or are present only in the presence/proximity to the headache attack.

The aims of this study were 1) to assess pain thresholds in trigeminal, cervical, and distal pain-free areas using different QST modalities in EM patients and 2) and correlate signs of sensitization with a) the interval from the last and the next headache attack and b) the clinical characteristic of headache and headache-related disability.

We hypothesized that 1) pain thresholds in trigeminal, cervical, and distal pain-free areas are reduced in EM patients during the 4 phases of the migraine cycle compared to healthy controls and 2) signs of sensitization would be correlated with a) the interval from the last and the next headache attack and b) the clinical characteristic of headache and headache-related disability.

METHOD

Design

This multicenter, cross-sectional, observational study was conducted in the Headache Center of Parma and Genova (Italy). Headache patients and healthy subjects (controls) were assessed between April 2019 and March 2020. This study was approved by ethic committees in the "Ligurian Region" (244/2018) and "Area Vasta Emilia-Nord" (18305/2019). All patients signed an informed consent form.

Population

Patients on the waiting lists to receive the first visit to the Headache Centers were invited to participate in this study. Men and women aged between 18 and 65 with EM (with and without aura) were recruited in the interictal, preictal, ictal, and postictal phases. Patients were excluded if they had:

1. any other primary or secondary headache;
2. any other neurologic, psychiatric, rheumatic, or systemic pathology with medical diagnosis;
3. received manual therapy in the cervical spine in the last 6 months;
4. received cervical anesthetic block or botulin injection in the last 6 months;
5. changed the prophylactic treatment in the last 3 months;
6. were unable to speak and understand Italian.

Control participants were recruited specifically for this study. They were defined as healthy subjects with a maximum of two headache episodes per year that did not fulfill the criteria for migraine or any other primary headache type with no family history of migraine or other primary headaches. The inclusion criteria for the control subjects were the same as the criteria used for migraine patients.

Procedure

The first screening was made by a telephone interview, and patients were excluded if they presented any signs of red flags[25] or reported at least one exclusion criteria. Healthy controls were recruited from university students, hospital staff, university staff, and the general population. During the examination, two therapists blinded to the subject's diagnosis, one for each recruitment center (S.D. and M.C.), gave all patients one questionnaire to complete, performed the QST assessment, and explained how to fulfill a diary where they had to record headache characteristics for the following four weeks. After four weeks from the first evaluation, headache patients were visited by a neurologist who performed a diagnosis of headache according to the International Headache

Classification Criteria [26]. A neurologist retrospectively assessed the diary. Patients were divided into four different subgroups according to the phase of the migraine cycle in which the first examination was performed:

- Interictal: No headache attack occurred in the 48 hours before or after the evaluation
- Preictal: Headache attack occurred in the 48 hours after the evaluation
- Ictal: Headache attack during the evaluation
- Postictal: Headache attack occurred in the 48 hours before the evaluation

Migraine patients who fulfill criteria to be included both in the preictal and postictal groups were included in the preictal group if the nearest attack was the one after the evaluation and in the postictal group if the nearest attack was the one before the evaluation (Figure 1).

Assessments

For each subject, general characteristics were assessed (Table 1). Patients used a daily updated diary recording the total use of drugs and the frequency, intensity, and duration of headache attacks. Moreover, the headache side, the percentage of patients with aura, and total years lived with the headache were recorded. For patients with headache during the assessment, the pain intensity during the current headache attack was recorded (Table 2).

Quantitative sensory testing (QST)

QST was performed from distal pain-free areas first, then the cervical area, and finally the trigeminal area (symptomatic side in patients with unilateral migraine; dominant side in patients with side/shift or bilateral migraine and in controls). The examiner was kept blinded to the presence of headache for as long as possible.

- *Static pressure pain threshold (sPPT):* Pressure pain thresholds to hand-held algometry (Somedic AB, Sweden), probe area 1cm^2, 30 kPa/s force increase)[27,28] were assessed over the: trigeminal area, upper cervical spine (left and right), lower cervical spine (left and

right); distal pain-free areas (second metacarpophalangeal joint of the dominant hand; tibialis anterior muscle of the dominant leg).

- *Dynamic pressure pain threshold (dPPT):* dPPT was assessed to evaluate pressure pain threshold to a dynamic algometry (constant force spring controlled from 550 g to 5300 g)[4,29] over the posterior aspect of the neck (left and right sides).
- *Mechanical pain threshold (MPT):* MPT was used to assess mechanical pain threshold to pinpricks stimulation (from 0.80g to 50.1g nylon filaments)[30] over the following areas: trigeminal area; distal pain-free areas (thenar eminence of the dominant hand).
- *Wind-up ratio (WUR*): the WUR was used to assess the temporal summation of mechanical pinprick pain (50.1 g nylon filament). The subject was instructed to give a pain rating (11-point Numeric Rating Scale) for the first and last stimulus of 10 stimuli. The difference between the pain rating of the ten stimuli series and the pain rating of the first stimulus was calculated[31]. WUR was assessed over the trigeminal area. A positive WUR was a sign of increased temporal summation.

Headache Disability Index (HDI)

HDI questionnaire was used to assess two components of the headache-related disability: the emotional headache-related disability (HDI-E); the physical headache-related disability (HDI-P). The higher the score, the higher the disability[32,33].

Detailed of the assessment procedure are reported in the supplemental material (Appendix 1).

Statistical analysis

After a pilot study was conducted to calculate the effect size, a sample size calculation was performed using G*Power 3.1: 166 patients were required for regression models and 96 patients for the correlations to achieve a medium effect size (f2: 0.15; r: 0.30) with an alpha level of 0.05 and

the desired power of 95% and 85%, respectively. In order to calculate the sample size in the regression model, 9 predictors were included (5 covariates and 4 dummy variables, one for each group comparison). Mean (standard deviation) or median (interquartile range) of QST results were presented among controls and patients at different phases of the migraine cycle. This analysis was the primary a priory analysis of these data. We used linear regression models to compare QST results of patients at specific migraine phases to controls while adjusting for possible confounders (gender, age, body mass index, use of preventive pharmacological therapy, and use of symptomatic drugs in the 24 hours before the evaluation). We made appropriate transformations when the normality assumption was not fulfilled (sPPTs, dPPTs, temporal MPT, and hand MPT results were log-transformed). Spearman partial correlations adjusted for age and headache frequency were assessed between QST results and time relative to the last or the next migraine attack in interictal, preictal, and postictal EM patients pooled together. As a sensitivity analysis, the Spearman partial correlations adjusted for age and headache frequency were assessed between QST results and time relative to the last/next migraine attack in each migraine phase separately (interictal EM, preictal EM, and postictal EM). Age-adjusted Spearman partial correlations were calculated between QST results and the pain intensity during the current headache attack, headache characteristics, and headache-related disability in ictal EM. Spearman partial correlations adjusted for age and the time relative to the last/next migraine attack were calculated between QST results and headache characteristics and headache-related disability in interictal, preictal, and postictal EM patients pooled together. Subjects with one or more missing values were excluded from the correlation analysis. Data were adjusted for age as previous studies found a high correlation between age and QST results[34,35] and for headache frequency as the underlying headache frequency of each individual is directly related to the probability that they would be observed in a certain headache phase. As QST results could change in the proximity to a headache attack[7,36] when assessing the correlation between QST and headache characteristics and headache-related disability in a group formed by interictal, preictal, and postictal EM patients, data were also adjusted for the time relative

to the last/next migraine attack. The threshold accepted for statistical significance of the results was $p<0.05$, and tests of statistical significance were two-tailed. As multiple between-group comparisons were conducted, a sensitivity analysis was performed to assess which comparison would remain significant using a more conservative threshold for statistical significance. The p-value was calculated by dividing 0.05 for the total number of comparisons performed (0.05/4= 0.013). Statistical analyses were performed using the SAS software (version 9.4).

RESULTS

After 557 subjects were initially relucted, 181 were included (Table 1), 135 EM patients (Table 2), and 46 controls (Figure 1).

Quantitative sensory testing

sPPT and MPT were lower (increased sensitivity) in the trigeminal area interictal (sPPT: 198.5(79.3) kPa; p=0.021; MPT: 12.6(15.7) g; p=0.001), preictal (sPPT: 200.6(71.6) kPa; p=0.033; MPT: 10.7(12.4) g; p<0.001), ictal (sPPT: 171.4(95.9) kPa; p<0.001; MPT: 7.3(12.0) g; p<0.001), and postictal EM (sPPT: 182.2(76.3) kPa; p=0.006; MPT: 10.1(14.9) g; p=0.001), compared to controls (sPPT: 238.3(73.8) kPa; MPT: 21.9(17.3) g). WUR was facilitated in ictal EM compared to controls (EM versus controls: mean (SD) 2.7(2.0) versus 1.4(1.8); p=0.004) with no differences between control and interictal, preictal, and postictal EM. Cervical sPPTs and dPPT were reduced in interictal (sPPT upper cervical spine: 420.5(176.7) kPa; p=0.031; sPPT lower cervical spine: 458.6(207.3) kPa; p=0.002; dPPT: 4826.5(2698.0) g; p<0.001), preictal (sPPT upper cervical spine: 389.3(133.4) kPa; p=0.006; sPPT lower cervical spine: 450.8(174.3) kPa; p=0.005; dPPT: 4184.2(2628.3) g; p<0.001), ictal (sPPT upper cervical spine: 379.9(205.6) kPa p=0.003; sPPT lower cervical spine: 436.3(271.1) kPa; p=0.001; dPPT: 3838.3(2638.7) g; p<0.001), and postictal EM (sPPT upper cervical spine: 385.5(131.6) kPa; p=0.020; sPPT lower cervical spine: 413.0(150.3) kPa; p=0.002; dPPT: 4679.6(2894.9) g; p=0.001), compared to controls (sPPT upper

cervical spine: 494.9(171.5) kPa; sPPT lower cervical spine: 586.9(210.8) kPa; dPPT: 7693.9 (2896.8) g). sPPT in the metacarpophalangeal joint of the dominant hand was lower in preictal EM compared to controls (sPPT: 248.8 (96.6) kPa versus 319.8(112.3) kPa; p=0.006;), with no other significant differences. MPT on the thenar eminence was lower in interictal (22.3(15.6) g; p=0.002), preictal (23.6(12.2) g; p=0.035), ictal (22.4(17.0) g; p=0.004), and postictal (24.2(18.8) g p=0.003) EM compared to controls (32.5(14.4) g). No significant differences were found in sPPT over tibialis anterior muscles between controls and interictal, preictal, ictal, and postictal EM (Table 3; Supplemental material Appendix 2). The majority of the between-group differences remain significant using a more conservative p-value (Table 3; Supplemental material Appendix 2).

Correlations

No significant correlations were found between time relative to the last/next migraine attack and quantitative sensory testing in preictal, interictal, postictal EM pooled together (Table 4). A more sensitive analysis reveals a significant positive correlation between distance from next headache attack and MPT over temporalis (r=0.45; p=0.005), sPPT over the upper cervical spine (r=0.36; p=0.029), sPPT over the lower cervical spine (r=0.35; p=0.031), and sPPT over tibialis anterior muscles (r=0.33; p=0.044) in preictal EM. A significant positive correlation was found between distance from next headache attack and sPPT over the upper cervical spine (r=0.34; p=0.048) and hand sPPT (r=0.35; p=0.042) in interictal EM (Table 5).

In ictal EM, a significant positive correlation was found between cervical dPPT and years with headache (r=0.42; p=0.024) and between WUR and HDI-P (r=0.38; p=0.040) and HDI-E questionnaires (r=0.53; p=0.003). A significant negative correlation was found between pain intensity during the current headache attack and sPPT in the metacarpophalangeal joint of the dominant hand (r=-0.37; p=0.050) and MPT on the thenar eminence (r=-0.50; p=0.007). No other significant correlations were found (Table 6).

In interictal, preictal, and postictal EM pooled together, a significant negative correlation was found

between WUR over temporalis and headache frequency (r=-0.23; p=0.022), headache intensity (r=-0.21; p=0.040), HDI-P (r=-0.29; p=0.003), and HDI-E questionnaires (r=-0.34; p=0.001), and a significant negative correlation between MPT over the thenar eminence and years lived with migraine (r=-0.25; p=0.011) and monthly usage of symptomatic drugs (r=-0.31; p=0.002). No other significant correlations were found (Table 7).

DISCUSSION

This study showed that patients with episodic migraine show signs of sensitization in the trigeminocervical area in all phases of the migraine cycle, with the most prominent sensitization in the ictal phase. In addition, signs of widespread sensitization were consistent in the preictal period and in the subgroups of patients with the longest disease duration and more usage of symptomatic drugs.

Quantitative Sensory Testing

Ictal EM vs. Controls

Trigeminal and Cervical Sensitization

Signs of sensitization were found in the trigeminal and cervical areas in ictal EM patients. The migraine attack is characterized by increased sensitization of second-order neurons in the trigeminocervical complex[37–40] that could be initiated either by nociceptive input from blood vessels and meninges afferents[40–43] or by descending input from higher diencephalon and brainstem areas[2,40,44–46]. As the trigeminocervical complex also converges ipsilateral and contralateral input from face and neck[47–50], the sensitization of those neurons could lead to secondary hyperalgesia (referred hyperalgesia) in the cervical and the face receptive field[11,51,52]. Increased modality-specific hyperalgesia in the trigeminal and cervical area in the ictal phase of the migraine cycle[8,9,23] can be interpreted as the behavioral consequence of the "activity-dependent central sensitization" of second-order neurons in the trigeminocervical complex[53]. To the author's knowledge, the present

study is the first to show facilitated temporal pain summation in the trigeminal area, specifically during the ictal phase. Temporal summation of pain in humans is suggested as the behavioral consequence of wind-up-like pain, as shown in animals[53,54]. As enhanced sensitization of post-synaptic receptors could mediate the facilitation of the temporal summation of pain [55], and during the ictal phase of the migraine cycle enhanced sensitization of second-order neurons in the spinal trigeminal nucleus has been observed[37,38], the mechanism involved in the facilitation of the temporal summation during the ictal phase could be an increase in central sensitization of e.g. second-order neurons in the spinal trigeminal nucleus.

Widespread Sensitization

The lower pain thresholds in the trigeminal and cervical area in the ictal EM patients could represent activity-dependent sensitization of the trigeminocervical complex in the brainstem and hyperalgesia in distal pain-free areas as a sign of activity-dependent sensitization of spinal cord neurons and higher cortical/subcortical brain areas[56–58]. The current study showed MPT hyperalgesia over the hand with no difference in sPPT contrasting previous studies showing widespread sPPT sensitization[8] and no difference in hand MPTs[24]. This difference may be explained by widespread ictal sensitization only in a subgroup of migraine patients[9], but often heterogeneous samples of migraine patients are lumped together. During the ictal phase of the migraine cycle, signs of widespread sensitization seem to occur only 2 hours after the headache phase begins[11] as another variable when studies are compared. The present study did not control the interval from the beginning of the headache phase.

Interictal EM vs. Controls

Trigeminal and Cervical Sensitization

The present results showed signs of sensitization to different stimulus modalities in the trigeminal and cervical area in interictal phase. The reductions in trigeminal sPPT are in accordance with

previous results[23], whereas trigeminal MPT reduction has not been reported[22,24,59]. Previous studies have recruited young migraine patients with short disease duration and low chronicity[59] or not matched controls for age and sex[24]. Moreover, differently from previous studies[22,24,59], importantly, we exclude controls with a family history of headache[17,60]. MPT was not the primary outcome in the previous studies[22,24,59], and the sample size calculations were not made to detect a difference in this outcome. To the authors' knowledge, the present study is the first to assess cervical mechanical pain thresholds in interictal EM patients controlling for previous and subsequent headache attacks, providing evidence of enhancing sensitization in the cervical area in this subgroup of patients in the trigeminocervical area. The enhanced sensitization observed interictally could be seen as the behavioral consequence of the "late-onset transcription-dependent central sensitization" of second-order neurons in the trigeminocervical complex[53].

Widespread Sensitization

In the interictal phases, only MPT over the hand was reduced, contrasting previous studies[22,24,59]. As a subgroup of migraine patients showed signs of ictal widespread sensitization[9], it is plausible that a similar percentage of subjects will show enhance interictal widespread sensitization compared to healthy controls, potentially explaining the heterogeneity with previous studies.

Peri-ictal EM vs. Controls

Trigeminal and Cervical Sensitization

The present data showed signs of increased sensitization of the trigeminocervical complex in preictal and postictal EM patients[8,23]. The enhanced sensitization of trigeminal and cervical areas observed in the preictal phase[7] could be evaluated as the behavioral consequence of the "activity-dependent central sensitization" of second-order neurons in the trigeminocervical complex mediated by the activation of diencephalon and brainstem areas[53]. This is supported by the observation that, during the preictal phase, diencephalon and brainstem areas increase their

activation[44,45,61,62] and their functional connectivity with the trigeminocervical complex[45], which also gradually increases its activity towards the next headache attack[63].

Widespread Sensitization

No consistent evidence of widespread sensitization was found from distal pain-free areas in postictal EM patients, contrasting previous studies[8,24]. However, the present data showed consistent evidence of increased widespread sensitization in preictal EM patients[7], with reduced mechanical pain threshold over the hand for two different sensory stimulus modalities. The preictal phase is characterized by activation of areas involved in pain processing and in descending modulation of nociceptive input[45,61,62,64] that could become dysfunctional, switching from being antinociceptive to pronociceptive leading to a migraine attack[2,65–67]. However, as other studies showed an enhance of endogenous analgesic mechanisms in the preictal phase[21,68], future longitudinal studies should assess the pain modulation (e.g. conditional pain modulation)[69,70] during the migraine cycle.

Quantitative sensory testing and interval between headache attacks

No correlations were observed between distance from the last migraine attack and QST results[21]. However, a positive correlation was found between trigeminal, cervical, and widespread sensitization and time to next headache attack in interictal and preictal EM, suggesting that threshold decrease towards the next headache attack when assessed in the preictal and ictal phases[7,36]. Importantly QST studies should control for the following headache attacks[14].

Quantitative sensory testing and clinical characteristics of headache and disability

A positive correlation was found between facilitation of the trigeminal temporal summation of pain and headache-related disability in ictal EM, suggesting that during the ictal phase of the migraine cycle, those migraine patients with a higher level of disability present a more pronounced sensitization of second-order neurons in the spinal trigeminal nucleus[71,72]. Moreover, a negative

correlation was found between pain intensity during the current headache attack and signs of widespread sensitization in ictal EM. Thus, patients with higher pain intensity during the current headache attack present more enhanced signs of widespread sensitization[73,74]

As opposed to the ictal phase, a negative correlation was found outside the headache phase between facilitation of the trigeminal temporal summation of pain and headache characteristic or headache-related disability. Thus, outside the headache attack, those migraine patients with a higher level of disability or worse clinical manifestation of headache present a less pronounced sensitization of second-order neurons in the spinal trigeminal nucleus[75]. Two possible mechanisms could explain these results. First, adaptive response of the trigeminal nociception pathway showing a reduction in its activity outside the headache phase could be present in those patients with worse clinical manifestations of migraine that experienced higher activation of the trigeminal nociception pathway during a migraine attack[76–78]. Secondly, as the acute headache attack was characterized by enhanced sensitization of neurons in the spinal trigeminal nucleus and in migraine patients repeated episodes may be associated with neuronal damage[79,80] and consequently changed activity of these neurons[63]. However, considering that these results are not supported in other studies[81,82], they should be interpreted with caution. Moreover, outside the headache attack, a positive correlation was found between signs of increased widespread sensitization and years lived with headache or the use of symptomatic drugs, suggesting that patients with longer disease duration and higher use of symptomatic drugs present more enhanced signs of widespread sensitization. Because the migraine attack could be considered a sensitizing nociceptive input [11], it is plausible that patients with a longer history develop a more widespread sensitization[7,9,83,84]. The correlation between higher use of symptomatic drugs and higher signs of widespread sensitization could be a result of reduced drug effect in patients with higher signs of sensitization[85,86], and hence patients increase their utilization. An alternative suggestion is that the drugs may enhance the sensitization [87,88].

Limitations

The population was recruited from a specialized headache center, and over half of the patients were excluded for age, concomitant pathologies, and concomitant diagnosis of other headache types. Thus, the external validity of these results should be interpreted with caution.

As migraine patients who fulfill criteria to be included in the preictal and postictal groups were included in one of the two groups according to the nearest attack, readers should be aware that some preictal migraine patients could also be in the postictal phase and vice versa.

The blindness of the assessor could not be maintained for the entire evaluation of every patient. QST in the trigeminal area was only assessed from one side to reduce the assessment duration, leading to a loss of blindness in patients with a unilateral headache on the non-dominant side. The assessor would be blinded regarding the headache type and phase.

Another limitation may be related to the assessment procedure. Even if the protocol we used to assess MPT was already applied in other EM patient studies[9,36,82], other authors used different procedures [22,24,59]. The differences in the protocol used can partially explain the heterogenicity of the results and made results hard to compare directly across different studies. Future studies should consider using a standardized protocol to assess MPT in the migraine population[35].

Finally, as the study has not a within-subjects design, comparisons between the different phases of the migraine cycle, thus, differences observed only between one migraine subgroup and healthy controls should not be interpreted as general differences between migraine subgroups.

Conclusion

In all phases of the migraine cycle, EM patients show signs of increased sensitization in the trigeminocervical area, with further facilitation approaching the headache attack. The temporal summation of pain was facilitated in the ictal phase. Moreover, during the ictal phase, the higher the headache-related disability, the more facilitated trigeminal temporal summation of pain. Signs of enhanced widespread sensitization were consistent in preictal EM patients and in the subgroups of EM patients with the longest disease duration and more usage of symptomatic drugs.

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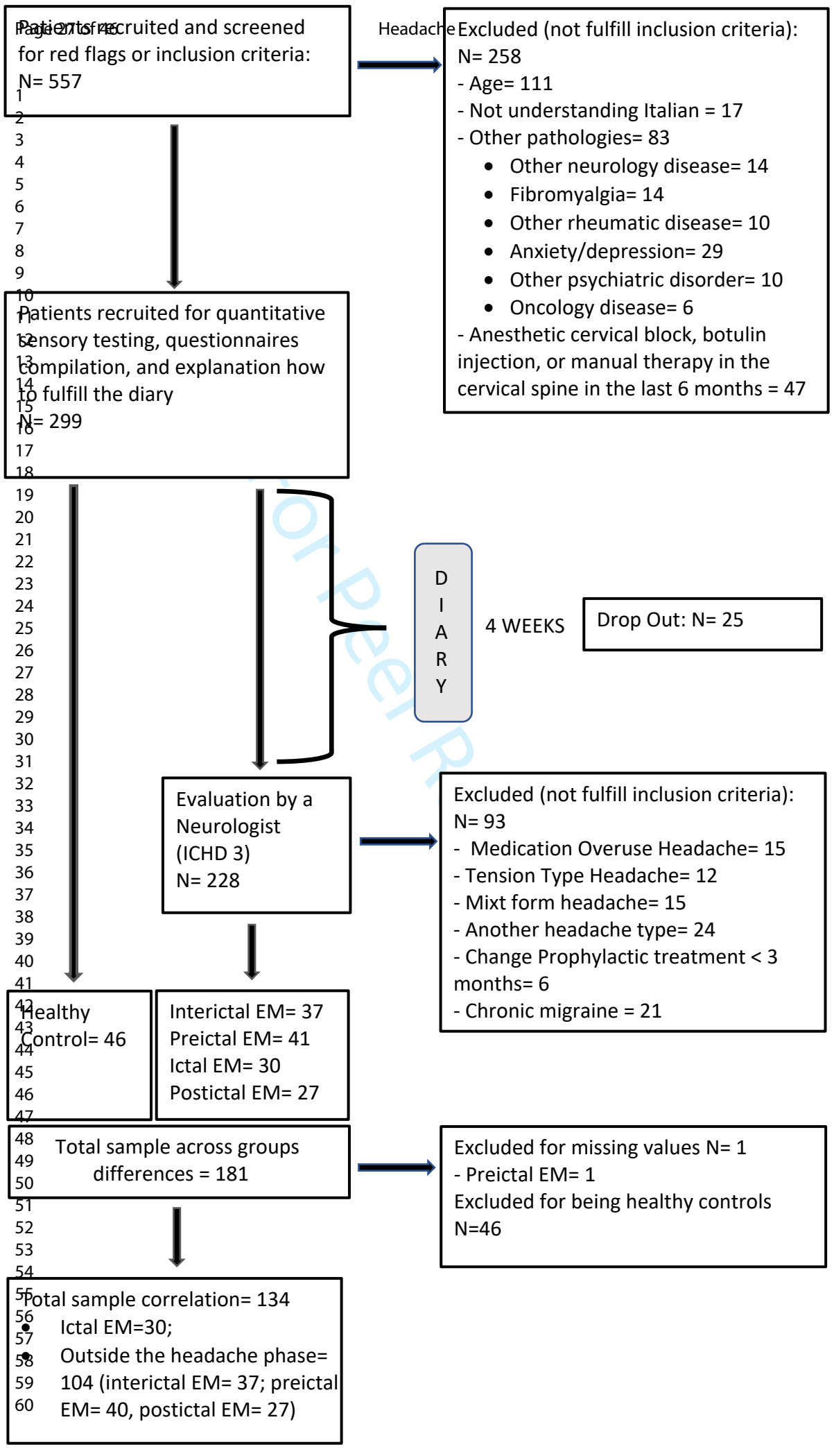
Patients recruited and screened for red flags or inclusion criteria:
N= 557
Headache
Excluded (not fulfill inclusion criteria):
N= 258
- Age= 111
- Not understanding Italian = 17
- Other pathologies= 83
• Other neurology disease= 14
• Fibromyalgia= 14
• Other rheumatic disease= 10
• Anxiety/depression= 29
• Other psychiatric disorder= 10
• Oncology disease= 6
- Anesthetic cervical block, botulin injection, or manual therapy in the cervical spine in the last 6 months = 47
Patients recruited for quantitative sensory testing, questionnaires compilation, and explanation how to fulfill the diary
N= 299
D I A R Y
4 WEEKS
Drop Out: N= 25
Evaluation by a Neurologist (ICHD 3)
N= 228
Excluded (not fulfill inclusion criteria):
N= 93
- Medication Overuse Headache= 15
- Tension Type Headache= 12
- Mixt form headache= 15
- Another headache type= 24
- Change Prophylactic treatment < 3 months= 6
- Chronic migraine = 21
Healthy Control= 46
Interictal EM= 37
Preictal EM= 41
Ictal EM= 30
Postictal EM= 27
Total sample across groups differences = 181
Excluded for missing values N= 1
- Preictal EM= 1
Excluded for being healthy controls
N=46
Total sample correlation= 134
• Ictal EM=30;
• Outside the headache phase= 104 (interictal EM= 37; preictal EM= 40, postictal EM= 27)

Table 1: General characteristic

	Control (n= 46)	EM interictal (n= 37)	EM preictal (n=41)	EM ictal (n=30)	EM postictal (n=27)
Age, Mean (SD)	37.7 (14.0)	38.3 (11.5)	40.4(13.6)	37.3(10.9)	35.9(12.0)
BMI, Mean (SD)	22.1(2.7)	22.7(3.6)	22.9(3.1)	23.3(4.3)	23.7(4.5)
Sex, N (%)					
Female	34(74%)	29(78%)	35(85%)	27(90%)	21(78%)
Male	12(26%)	8(22%)	6(15%)	3 (10%)	6(22%)
Dominant side, N (%)					
Right	44(96%)	34(92%)	39(95%)	28(93%)	26(96%)
Left	2(4%)	3(8%)	2(5%)	2(7%)	1(4%)
Menstrual Cycle, N (%)					
No	21(46%)	14(38%)	17(42%)	9(30%)	9(33%)
Yes	25(54%)	23(62%)	24(59%)	21(70%)	18(67%)
Distance from last first day of menstrual cycle, Mean (SD)	17.2(14.9)	16.8(14.5)	16.4(17.1)	18.5(16.9)	15.6(15.)
Use of symptomatic drugs in the last 24 hours, N (%)					
No	45(98%)	34(92%)	36(88%)	22(73%)	16(59%)
Yes	1(2%)	3(8%)	5(12%)	8(27%)	11(41%)
Use of prophylactic therapy, N (%)					
No	48 (100%)	33(89%)	32(78%)	29(97%)	24(89%)
Yes	0(0%)	4(10%)	9(21%)	1(3%)	3(11%)

BMI: body mass index; EM: episodic migraine; N: number; SD: standard deviation

	(n= 37)	(n=41)	(n=30)	(n=27)
Headache type, N (%)				
MwoA	31(84%)	35(85%)	28(93%)	26(96%)
MwA	6(16%)	6(17%)	2(7%)	1(4%)
Headache side, N (%)				
Bilateral	19(52%)	28(68%)	20(66%)	12(45%)
Left	5(13%)	5(12%)	5(17%)	6(22%)
Right	8(22%)	3(8%)	2(7%)	2(7%)
Side shift	5(13%)	5(12%)	3(10%)	7(26%)
Time from last headache attack, mean hours (SD)	276.9(211.9)	202.9(226.7)	0	21.00(12.8)
Time from next headache attack, mean hours (SD)	226.8(192.0)	18.6(10.5)	0	147.2(159.2)
Pain intensity during the current headache attack, mean NPRS 0-10 (SD)	0	0	3.7(2.3)	0
Years with headache, mean years (SD)	18.3(13.6)	19.2(14.0)	18.5(13.9)	18.4(12.6)
DIARY				
Frequency, mean day/ four weeks (SD)	5.0(3.1)	7.2(2.8)	8.3(3.4)	7.6(3.7)
Duration, mean hours/day (SD)	7.1(5.8)	7.1(5.2)	7.1(3.5)	7.1(4.8)
Intensity, mean NPRS 0-10 (SD)	5.8(1.5)	5.8(1.6)	5.3(1.9)	5.9(1.9)
Drugs, mean number of tablets / four weeks (SD)	3.7(3.4)	5.6(3.6)	5.0(4.3)	6.6(5.4)
HDI – P, mean (SD)	21.7(9.9)	25.3(11.9)	21.9(8.0)	23.4(10.1)
HDI – E, mean (SD)	17.6(9.3)	22.8(12.1)	19.1(10.8)	19.0(11.2)

Table 2: Headache characteristic

EM: episodic migraine: HDI-P: Headache disability index physical; HDI-E: Headache disability index Emotional; MwA: migraine with aura; MwoA: migraine without aura; NPRS: numeric pain rating scale; N: number; SD: standard deviation;

Table 3: Linear regression models using QST results as dependent variables and 9 predictors: gender, age, BMI, use of preventive pharmacological therapy, and use of symptomatic drugs in the 24 hours before the evaluation were first included in the models as covariate then four dummy variables (controls against EM patients in each phase) were included.

	Control(N=46)	EM Interictal(N=37)	EM preictal(N=41)	EM ictal(N=30)	EM postictal(N=27)
TRIGEMINAL AREA					
sPPT temporalis†, mean kPa (SD)	238.3(73.8)	198.5(79.3) **B=-0.21 p=0.021***	200.6(71.6) **B=-0.19 p=0.033***	171.4(95.9) **B=-0.38 p<0.001*#**	182.2(76.3) **B=-0.30 p=0.006*#**
MPT temporalis†, mean g (SD)	21.9(17.3)	12.6(15.7) **B=-0.90 p=0.001*#**	10.7(12.4) **B=-0.97 p<0.001*#**	7.3(12.0) **B=-1.38 p<0.001*#**	10.1(14.9) **B=-1.09 p=0.001*#**
WUR temporalis, mean (SD)	1.4(1.8)	1.7(1.6) B=0.042 p=0.371	1.8(2.5) B=0.59 p=0.218	2.7(2.0) **B=1.51 p=0.004*#**	1.7(2.6) B=0.075 p=0.196
CERVICAL AREA					
sPPT UCS total†, mean kPa (SD)	494.9(171.5)	420.5 (176.7) **B=-0.19 p=0.031***	389.3(133.4) **B=-0.24 p=0.006*#**	379.9(205.6) **B=-0.29 p=0.003*#**	385.5 (131.6) **B=-0.24 p=0.020***
sPPT LCS total†, mean kPa (SD)	586.9(210.8)	458.6(207.3) **B=-0.27 p=0.002*#**	450.8(174.3) **B=-0.25 p=0.005*#**	436.3(271.1) **B=-0.33 p=0.001*#**	413.0(150.3) **B=-0.34 p=0.002*#**
dPPT total† mean g (SD)	7693.9(2896.8)	4826.5(2698.0) **B=-0.51 p<0.001*#**	4184.2(2628.3) **B=-0.68 p<0.001*#**	3838.3(2638.7) **B=-0.71 p<0.001*#**	4679.6(2894.9) **B=-0.57 p<0.001*#**
DISTAL PAIN-FREE AREAS					
sPPT second MCP†, mean kPa (SD)	319.8(112.3)	278.0(110.6) B=-0.15 p=0.089	248.8(96.6) **B=-0.24 p=0.006*#**	280.0(118.5) B=-0.13 p=0.159	299.3(125.8) B=-0.07 p=0.519
MPT thenar eminence†, mean g (SD)	32.5(14.4)	22.3(15.6) **B=-0.55 p=0.002*#**	23.6(12.2) **B=-0.37 p=0.035***	22.4(17.0) **B=-0.57 p=0.004*#**	24.2(18.8) **B=-0.64 p=0.003*#**
sPPT tibialis muscle†, mean kPa (SD)	407.8(183.0)	391.2(191.6) B=-0.03 p=0.737	366.6(140.4) B=-0.03 p=0.767	358.7(200.1) B=-0.10 p=0.381	356.9(166.4) B=-0.09 p=0.447

BMI: body mass index; dPPT: Dynamic pressure pain threshold; EM: Episodic migraine; g: grams; LCS: lower cervical spine; MPT: Mechanical pain threshold; MCP: Metacarpophalangeal; QST: quantitative sensory testing; sPPT: Static pressure pain threshold; UCS: upper cervical spine; kPa: kilopascal; WUR: wind up ratio *: significant at p<0.05 vs. Control; #: significant at p<0.013 vs. Control; †= data were log-transformed for statistical analysis;

Table 4: Spearman Partial correlations adjusted for age and headache frequency in preictal, interictal, postictal EM polled together

	sPPT temporalis	MPT temporalis	WUR temporalis	sPPT upper cervical spine (total)	sPPT lower cervical spine (total)	dPPT cervical (total)	sPPT second MCP	MPT thenar eminence	sPPT tibialis muscle
Time from last headache attack									
r	0.14	0.16	-0.17	0.02	0.08	-0.10	-0.17	-0.06	0.08
p	0.161	0.106	0.088	0.842	0.444	0.309	0.097	0.540	0.407
Time from next headache attack									
r	0.07	0.03	-0.09	0.15	0.13	0.19	0.18	-0.05	0.10
p	0.516	0.747	0.372	0.124	0.180	0.050	0.064	0.594	0.323

dPPT: Dynamic pressure pain threshold; MPT: Mechanical pain threshold; MCP: Metacarpophalangeal; sPPT: Static pressure pain threshold; WUR: wind up ratio *: significant ant p<0.05

Table 5: Spearman Partial correlations adjusted for age and headache frequency in preictal, interictal, and postictal EM

	sPPT temporalis	MPT temporalis	WUR temporalis	sPPT upper cervical spine (total)	PPT lower cervical spine (total)	dPPT cervical (total)	sPPT second MCP	MPT thenar eminence	sPPT tibialis muscle
Preictal EM									
Distance from last headache attack									
r	0.15	0.22	-0.31	0.12	0.16	0.00	0.08	-0.02	0.07
p	0.374	0.176	0.057	0.476	0.339	0.998	0.652	0.925	0.680
Distance from next headache attack									
r	0.24	**0.45**	-0.06	**0.36**	**0.35**	0.15	-0.08	-0.08	**0.33**
p	0.156	**0.005***	0.706	**0.029***	**0.031***	0.384	0.652	0.625	**0.044***
Interictal EM									
Distance from last headache attack									
r	0.11	0.06	0.16	-0.04	-0.10	-0.23	-0.27	-0.14	0.01
p	0.546	0.751	0.374	0.804	0.555	0.185	0.123	0.410	0.961
Distance from next headache attack									
r	0.28	-0.05	-0.03	0.24	**0.34**	0.20	**0.35**	0.21	0.29
p	0.101	0.767	0.875	0.168	**0.048***	0.257	**0.042***	0.235	0.092
Postictal EM									
Distance from last headache attack									
r	0.03	0.02	-0.06	-0.28	-0.27	-0.07	-0.39	-0.06	-0.22
p	0.876	0.943	0.769	0.177	0.200	0.740	0.056	0.762	0.282
Distance from next headache attack									
r	0.18	0.02	-0.35	-0.00	-0.04	-0.05	0.06	0.17	0.09
p	0.383	0.925	0.086	0.996	0.856	0.819	0.782	0.415	0.667

dPPT: Dynamic pressure pain threshold; EM: episodic migraine; MPT: Mechanical pain threshold; MCP: Metacarpophalangeal; sPPT: Static pressure pain threshold; WUR: wind up ratio *: significant ant $p<0.05$

Table 6: Age-adjusted Spearman partial correlations in ictal EM

	sPPT temporalis	MPT temporalis	WUR temporalis	sPPT upper cervical spine (total)	sPPT lower cervical spine (total)	dPPT cervical (total)	sPPT second MCP	MPT thenar eminence	sPPT tibialis muscle
Years with headache									
r	0.17	-0.20	0.16	0.03	0.10	**0.42**	0.21	0.20	0.32
p	0.369	0.309	0.400	0.862	0.609	**0.024***	0.273	0.297	0.086
Frequency									
r	-0.02	-0.18	0.09	-0.02	-0.09	0.02	0.09	0.04	0.13
p	0.912	0.349	0.663	0.906	0.643	0.929	0.657	0.822	0.507
Duration									
r	-0.27	-0.09	0.25	-0.27	-0.15	-0.07	0.02	0.23	-0.13
p	0.152	0.659	0.201	0.165	0.446	0.731	0.939	0.236	0.490
Intensity									
r	-0.00	-0.23	0.18	-0.10	-0.12	-0.18	-0.14	-0.31	-0.09
p	0.896	0.221	0.335	0.595	0.528	0.344	0.469	0.100	0.632
Drugs									
r	0.11	0.01	-0.05	0.07	0.06	0.02	-0.00	-0.08	0.04
p	0.562	0.968	0.811	0.707	0.741	0.925	0.988	0.688	0.830
HDI-P									
r	-0.16	-0.05	**0.38**	-0.05	-0.08	-0.19	0.04	-0.20	-0.23
p	0.399	0.810	**0.040***	0.808	0.691	0.331	0.841	0.290	0.223
HDI-E									
r	-0.23	-0.11	**0.53**	-0.32	-0.25	-0.27	-0.08	-0.12	-0.29
p	0.236	0.556	**0.003***	0.090	0.198	0.161	0.666	0.550	0.122
Pain intensity during the current headache attack									
r	-0.16	-0.26	0.15	-0.04	-0.27	-0.10	**-0.37**	**-0.49**	-0.03
p	0.404	0.176	0.429	0.847	0.165	0.619	**0.050***	**0.007***	0.875

dPPT: Dynamic pressure pain threshold; HDI-P: Headache disability index physical; HDI-E: Headache disability index Emotional MPT: Mechanical pain threshold; MCP: Metacarpophalangeal; sPPT: Static pressure pain threshold; WUR: wind up ratio *: significant ant p<0.05

Table 7: Spearman Partial Correlations adjusted for age, time from last headache attack, and time from next headache attack in preictal, interictal, postictal EM polled together

	sPPT temporalis	MPT temporalis	WUR temporalis	sPPT upper cervical spine (total)	sPPT lower cervical spine (total)	dPPT cervical (total)	sPPT second MCP	MPT thenar eminence	sPPT tibialis muscle
Years with headache									
r	0.06	-0.14	0.02	0.08	0.07	-0.05	-0.09	**-0.25**	0.02
p	0.575	0.180	0.821	0.407	0.520	0.614	0.396	**0.011***	0.870
Frequency									
r	0.10	0.09	**-0.23**	0.11	0.18	-0.02	-0.03	-0.10	0.13
p	0.347	0.377	**0.022***	0.293	0.069	0.877	0.780	0.337	0.213
Duration									
r	-0.02	-0.06	-0.04	-0.14	-0.10	-0.11	-0.07	-0.15	-0.01
p	0.824	0.543	0.706	0.165	0.346	0.273	0.461	0.131	0.920
Intensity									
r	-0.11	-0.02	**-0.21**	-0.16	-0.11	-0.02	-0.06	-0.07	-0.08
p	0.266	0.878	**0.040***	0.103	0.269	0.823	0.548	0.493	0.441
Drugs									
r	0.06	-0.05	-0.10	0.05	0.11	0.08	0.05	**-0.31**	0.05
p	0.585	0.631	0.317	0.617	0.259	0.430	0.644	**0.002***	0.605
HDI-P									
r	0.09	0.03	**-0.29**	-0.05	0.02	-0.03	-0.05	-0.06	0.02
p	0.376	0.735	**0.003***	0.617	0.875	0.787	0.593	0.568	0.870
HDI-E									
r	-0.08	0.07	**-0.34**	-0.13	-0.07	-0.06	-0.14	-0.02	-0.08
p11	0.448	0.486	**0.001***	0.185	0.515	0.553	0.178	0.882	0.448

dPPT: Dynamic pressure pain threshold; HDI-P: Headache disability index physical; HDI-E: Headache disability index Emotional MPT: Mechanical pain threshold; MCP: Metacarpophalangeal; sPPT: static pressure pain threshold; WUR: wind up ratio *: significant ant p<0.05

5Appendix 1: assessment

General characteristic and Headache characteristic

For each subject, the following variables were assessed: sex, age, body mass index (BMI), dominant side, presence of menstrual cycle, distance from the evaluation and the last first day of the menstrual cycle, use of symptomatic drugs in the 24 hours before the evaluation, and use of prophylactic drugs. To assess the characteristic of headache attacks, we used a daily updated diary where patients recorded the frequency of headache attacks (days in four weeks), the intensity of the headache attacks on an 11-points numerical pain rate scale (NPRS; 0: no pain, 10: the maximum pain), the mean duration of headache attack (mean hours for attack), total use of drugs (number of symptomatic drugs in four weeks). Moreover, the headache side, the percentage of patients with aura, and total years lived with the headache were recorded. For those patients with headache during the assessment, the pain intensity during the current headache attack was recorded on an 11-points numerical pain rate scale (NPRS; 0: no pain, 10: the maximum pain).

Quantitative sensory testing

Static pressure pain threshold (sPPT)

An electronic algometer with a probe of 1 cm2 (Somedic AB, Farsta, Sweden) was used to determine sPPT, i.e., the minimal amount of pressure where a sensation of pressure first changes to pain. Subjects were instructed to press the algometer ‘‘stop button’’ as soon as the pressure resulted in the first sensation of pain. The pressure was increased at a rate of approximately 30 kPa/s. The mean of three trials on each point was calculated and used for the analysis. A 30 second resting period was allowed between trials to avoid temporal summation[1]. Pressure algometry has high reliability (test-retest reliability (TR-R) = 0.88; interobserver reliability (IO-R) = 0.84)[2] and was already used to assess pressure pain threshold in patients with migraine[3,4]. sPPTs were from four different areas

- Trigeminal area: sPPTs were assessed over the anterior, middle, and posterior columns of the temporalis muscles, and the mean of these three points was calculated. As previous studies have shown no side-to-side differences in sPPT over the trigeminal area in EM patients with unilateral migraine[4,5], the symptomatic side was assessed for patients with unilateral migraine. In contrast, the dominant side was assessed in patients with side/shift or bilateral migraine and in healthy controls.
- Upper cervical spine: Upper cervical spine: sPPTs were over four areas, corresponding to the right / left posterior arch of the atlas (C1) and right / left articular pillar of the axis (C2). The mean of the two different points for each side was calculated: upper left cervical spine and upper right cervical spine.
- Lower cervical spine: sPPTs were assessed over four areas, corresponding to the right / left articular pillar of C4 and right / left articular pillar of C6. The mean of the two different points for each side was calculated: lower left cervical spine and lower right cervical spine.
- Distal pain-free areas: sPPTs were assessed over the second metacarpophalangeal joint of the dominant hand and tibialis anterior muscle of the dominant leg.

Dynamic pressure pain threshold (dPPT)

A roller pressure algometer was used to evaluate dPPT (Aalborg University, Denmark). The roller pressure algometer consisted of a wheel through which the assessor could apply eight different rollers, with a fixed load level of 500 g, 700 g, 850 g, 1350 g, 1500 g, 2200 g, 3300, and 5300 g, respectively. The wheel, made of hard plastic, has a diameter of 35 mm and a width of 10 mm. The assessor maintained a constant pressure while the roller was moving at a speed of approximately 0.5cm/sec. The track of the roller was around 100 mm, crossing over the posterior aspect of the neck approximately 20 mm lateral to the spinal process from C7 to C2 vertebral segment (caudal to cranial), with a total dynamically-stimulated area of 10 mm x 100 mm. The assessment was repeated two times on each side of the neck. The second stimulation on the same side was applied

when the pain provoked by the first stimulation disappeared. The load level of the roller where the dynamic pressure was first perceived as painful for the two stimuli was defined as the dPPT. A set of roller pressure algometers were considered valid and reliable tools to evaluate deep dynamic pressure sensitivity[6] with high intrarater reliability (intraclass correlation coefficient = 0.88)[7] and were previously used to assess dynamic pressure sensitivity in patients with migraine[8].

Mechanical pain threshold (MPT)

A set of weight-calibrated pinpricks (Aalborg University, Aalborg, Denmark) was used to assess mechanical pain threshold (MPT) to pinpricks stimulation. The pinprick set consists of seven metal probes (fixed diameter tip of 0.6 mm) with different force applications: 0.8g, 1.6g, 3.2g, 6.4g, 12.8g, 25.6g, and 50.1g. Starting from the lightest weight, each pin was applied for 2s in the area until the subject felt that the sensation changed from "an innocuous prodding" to a "sharp pricking". Two repeated stimulations were performed with each pinprick. The weight of the pinprick, which induced the "sharp pricking" for both stimuli, was defined as the pain threshold [9]. A set of weight-calibrated pinpricks were considered to have high reliability (TR-R= 0.80; IO-R= 0.80)[2] and were already used to assess mechanical pain threshold in patients with migraine[10,11]. MPT was assessed from two different areas:

- Trigeminal area: MPT was assessed over the anterior column of the temporalis muscles. As for the sPPT, the symptomatic side was assessed for patients with unilateral migraine, while the dominant side was assessed in patients with side/shift or bilateral migraine or healthy controls.
- Distal pain-free area: MPT was assessed over the thenar eminence of the dominant hand.

Wind-up ration (WUR)

The WUR was calculated to assess the temporal summation of mechanical pain. WUR was measured by comparing the perceived magnitude of pain from a single pinprick stimulus (50.1g) with that of a series of 10 pinprick stimuli of the same force delivered a 1/s rate within an area of 1

cm^2. The subject was instructed to give a pain rating for the first single stimulus and the last stimulus of 10 stimuli using an 11-point Numeric Rating Scale (NRS11). The difference between the pain rating of the ten stimuli series and the pain rating of the first stimulus was calculated so that a positive WUR was a sign of increased temporal summation of mechanical pain[12]. This method exhibited good reliability (TR-R= 0.67; IO-R= 0.56)[2] and was previously used in migraine patients [11,13]. WUR was assessed over the anterior column of the temporalis muscles. The symptomatic side was assessed for patients with unilateral migraine, while the dominant side was assessed in patients with side/shift or bilateral migraine and in healthy controls.

QST protocol was performed in a standardized manner. The distal pain-free areas were first assessed, then the cervical area, and finally the trigeminal area. This standard procedure permit to keep the examiner blinded to the presence of headache as long as possible.

Questionnaires

Headache disability index (HDI)

HDI questionnaire was used to assess the headache-related disability. This questionnaire uses 25 items that investigate the perceived impact of headache on emotional functioning and daily life activities and provides a 0-100 total score, with a higher score indicating a high level of disability. Thirteen items assess the emotional burden (HDI-E, maximum score: 52), whereas the remaining 12 items assess the physical burden (HDI-P, maximum score: 48). This questionnaire has demonstrated high reliability (TR-R= 0.93-0.95)[14] and was already used to assess disability in patients with migraine [15,16]

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Appendix 2: Linear regression models using QST results as dependent variables and 9 predictors: gender, age, BMI, use of preventive pharmacological therapy, and use of symptomatic drugs in the 24 hours before the evaluation were first included in the models as covariate then four dummy variables (controls against EM patients in each phase) were included.

	Control(N=46)	EM Interictal(N=37)	EM preictal(N=41)	EM ictal(N=30)	EM postictal(N=27)
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sPPT temporalis†, mean kPa (SD)	238.3(73.8)	198.5(79.3) **B=-0.21 p=0.021***	200.6(71.6) **B=-0.19 p=0.033***	171.4(95.9) **B=-0.38 p<0.001*#**	182.2(76.3) **B=-0.30 p=0.006*#**
MPT temporalis†, mean g (SD)	21.9(17.3)	12.6(15.7) **B=-0.90 p=0.001*#**	10.7(12.4) **B=-0.97 p<0.001*#**	7.3(12.0) **B=-1.38 p<0.001*#**	10.1(14.9) **B=-1.09 p=0.001*#**
WUR temporalis, mean (SD)	1.4(1.8)	1.7(1.6) B=0.042 p=0.371	1.8(2.5) B=0.59 p=0.218	2.7(2.0) **B=1.51 p=0.004*#**	1.7(2.6) B=0.075 p=0.196
CERVICAL AREA					
sPPT UCS total†, mean kPa (SD)	494.9(171.5)	420.5 (176.7) **B=-0.19 p=0.031***	389.3(133.4) **B=-0.24 p=0.006*#**	379.9(205.6) **B=-0.29 p=0.003*#**	385.5 (131.6) **B=-0.24 p=0.020***
sPPT UCS left†, mean kPa (SD)	246.1(92.5)	204.3 (82.1) **B=-0.19 p=0.030***	188.8(65.6) **B=-0.25 p=0.006*#**	187.6(97.4) **B=-0.27 p=0.006*#**	194.3(68.8) **B=-0.22 p=0.043***
sPPT USC right†, mean kPa (SD)	248.8(83.3)	216.2(97.1) **B=-0.18 p=0.041***	200.6(77.9) **B=-0.23 p=0.010*#**	192.3 (110.6) **B=-0.30 p=0.002*#**	191.2 (65.9) **B=-0.27 p=0.012*#**
sPPT LCS total†, mean kPa (SD)	586.9(210.8)	458.6(207.3) **B=-0.27 p=0.002*#**	450.8(174.3) **B=-0.25 p=0.005*#**	436.3(271.1) **B=-0.33 p=0.001*#**	413.0(150.3) **B=-0.34 p=0.002*#**
sPPT LCS left†, mean kPa (SD)	293.4(102.2)	224.2(101.1) **B=0.29 p=0.001*#**	217.2 (83.0) **B=-0.30 p=0.001*#**	217.2(135.2) **B=-0.33 p=0.001*#**	199.4 (68.0) **B=-0.37 p=0.001*#**
sPPT LCS right†, mean kPa (SD)	293.5(112.0)	233.4(108.6) **B=-0.24 p=0.006*#**	233.6(94.9) **B=-0.21 p=0.022***	219.1(137.2) **B=-0.32 p=0.001*#**	213.2(89.8) **B=-0.31 p=0.004*#**
dPPT total† mean g (SD)	7693.9(2896.8)	4826.5(2698.0) **B=-0.51 p<0.001*#**	4184.2(2628.3) **B=-0.68 p<0.001*#**	3838.3(2638.7) **B=-0.71 p<0.001*#**	4679.6(2894.9) **B=-0.57 p<0.001*#**
dPPT left† mean g (SD)	3850.0(1425.2)	2498.7(1452.3) **B=-0.49 p<0.001*#**	2106.1(1344.6) **B=-0.69 p<0.001*#**	1873.3 (1285.3) **B=-0.82 p<0.001*#**	2392.6(1454.6) **B=-0.55 p<0.001*#**
dPPT right† mean g (SD)	3884.8(1550.3)	2402.7(1386.3) **B=-0.53 p<0.001*#**	2085.4(1412.9) **B=-0.73 p<0.001*#**	1968.3(1430.7) **B=-0.81 p<0.001*#**	2287.0(1496.6) **B=-0.66 p<0.001*#**
DISTAL PAIN-FREE AREAS					
sPPT second MCP†, mean kPa (SD)	319.8(112.3)	278.0(110.6) B=-0.15 p=0.089	248.8(96.6) **B=-0.24 p=0.006*#**	280.0(118.5) B=-0.13 p=0.159	299.3(125.8) B=-0.07 p=0.519
MPT thenar eminence†, mean g (SD)	32.5(14.4)	22.3(15.6) **B=-0.55 p=0.002*#**	23.6(12.2) **B=-0.37 p=0.035***	22.4(17.0) **B=-0.57 p=0.004*#**	24.2(18.8) **B=-0.64 p=0.003*#**
sPPT tibialis muscle†, mean kPa (SD)	407.8(183.0)	391.2(191.6) B=-0.03 p=0.737	366.6(140.4) B=-0.03 p=0.767	358.7(200.1) B=-0.10 p=0.381	356.9(166.4) B=-0.09 p=0.447

BMI: body mass index; dPPT: Dynamic pressure pain threshold; EM: Episodic migraine; g: grams; LCS: lower cervical spine; MPT: Mechanical pain threshold; MCP: Metacarpophalangeal; QST: quantitative sensory testing; sPPT: Static pressure pain threshold; UCS: upper cervical spine; kPa: kilopascal; WUR: wind up ratio *: significant at p<0.05 vs. Control; #: significant at p<0.013 vs. Control; †= data were log-transformed for statistical analysis;

STROBE Statement—checklist of items that should be included in reports of observational studies

YOU MUST NOTE THE PAGE NUMBER WHERE EACH ITEM IS REPORTED INSIDE THE BRACKETS [ ]. IF NOT APPLICABLE WRITE N/A

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **[1]**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **[1,2]**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **[2,3]**
Objectives	3	State specific objectives, including any prespecified hypotheses **[3]**
Methods		
Study design	4	Present key elements of study design early in the paper **[3]**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **[3,4]**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **[N/A]** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **[N/A.]** *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **[4]**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **[N/A]** *Case-control study*—For matched studies, give matching criteria and the number of controls per case **[N/A]**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **[5,6, Supplemental material Appendix 1]**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **[5,6; Supplemental material Appendix 1]**
Bias	9	Describe any efforts to address potential sources of bias **[6,7]**
Study size	10	Explain how the study size was arrived at **[6,7]**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **[5-7, Supplemental material Appendix 1]**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **[6,7]**
		(*b*) Describe any methods used to examine subgroups and interactions **[6,7]**
		(*c*) Explain how missing data were addressed **[6,7]**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **[N/A]** *Case-control study*—If applicable, explain how matching of cases and controls was addressed **[N/A]** *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy **[6,7]**
		(*e*) Describe any sensitivity analyses **[6,7]**

Continued on next pag

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **[8, Figure 1]**
		(b) Give reasons for non-participation at each stage **[8, Figure 1]**
		(c) Consider use of a flow diagram **[Figure 1]**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **[Table 1, Table 2]**
		(b) Indicate number of participants with missing data for each variable of interest **[Figure 1]**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **[N/A]**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time **[N/A]**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure **[N/A]**
		Cross-sectional study—Report numbers of outcome events or summary measures **[8-10 Figure 1, Table 3-7, Supplemental material Appendix 2]**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **[8-10, Table 3-7, , Supplemental material Appendix 2]**
		(*b*) Report category boundaries when continuous variables were categorized **[Table 3-7]**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **[N/A]**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **[8-10 Table 5]**
Discussion		
Key results	18	Summarise key results with reference to study objectives **[10,15]**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **[14,15]**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **[10-14]**
Generalisability	21	Discuss the generalisability (external validity) of the study results **[14]**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **[Title file pag. 2]**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Once you have completed this checklist, please save a copy and upload it as part of your submission. When requested to do so as part of the upload process, please select the file type: *Checklist*. You will NOT be able to proceed with submission unless the checklist has been uploaded. Please DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.